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Genetic and Biochemical Characterization of OXA-405, an OXA-48-Type Extended-Spectrum β-Lactamase without Significant Carbapenemase Activity

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The epidemiology of carbapenemases worldwide is showing that OXA-48 variants are becoming the predominant carbapenemase type in *Enterobacteriaceae* in many countries. However, not all OXA-48 variants possess significant activity toward carbapenems (e.g., OXA-163). Two *Serratia marcescens* isolates with resistance either to carbapenems or to extended-spectrum cephalosporins were successively recovered from the same patient. A genomic comparison using pulsed-field gel electrophoresis and automated Rep-PCR typing identified a 97.8% similarity between the two isolates. Both strains were resistant to penicillins and first-generation cephalosporins. The first isolate was susceptible to expanded-spectrum cephalosporins, was resistant to carbapenems, and had a significant carbapenemase activity (positive Carba NP test) related to the expression of OXA-48. The second isolate was resistant to expanded-spectrum cephalosporins, was susceptible to carbapenems, and did not express a significant imipenemase activity, (negative for the Carba NP test) despite possessing a *bla*$_{\text{OXA-48}}$-type gene. Sequencing identified a novel OXA-48-type β-lactamase, OXA-405, with a four-amino-acid deletion compared to OXA-48. The *bla*$_{\text{OXA-405}}$ gene was located on a ca. 46-kb plasmid identical to the prototype IncL/M *bla*$_{\text{OXA-48}}$-carrying plasmid except for a ca. 16.4-kb deletion in the *tra* operon, leading to the suppression of self-conjugation properties. Biochemical analysis showed that OXA-405 has clavulanic acid-inhibited activity toward expanded-spectrum activity without significant imipenemase activity. This is the first identification of a successive switch of catalytic activity in OXA-48-like β-lactamases, suggesting their plasticity. Therefore, this report suggests that the first-line screening of carbapenemase producers in *Enterobacteriaceae* may be based on the biochemical detection of carbapenemase activity in clinical settings.

Ambler class D β-lactamases (oxacillinases) are widely disseminated among clinically relevant Gram-negative bacteria (1). They exhibit a high degree of diversity of hydrolysis activity ranging from narrow to broad-spectrum hydrolysis activity toward β-lactams (1). Among the class D β-lactamases, several enzymes hydrolyze carbapenems. Most carbapenem-hydrolyzing class D β-lactamases (CHDLs) are from *Acinetobacter* spp. (e.g., OXA-23, OXA-40, OXA-58, OXA-143) (2, 3), whereas OXA-48-type enzymes are identified in *Enterobacteriaceae* only (4). The OXA-48-derived CHDLs have initially been identified in Turkey (5), first in *Klebsiella pneumoniae* and then in other enterobacterial species (4). The known OXA-48 variants are currently as follows: (i) OXA-162, identified from *K. pneumoniae* isolates in Turkey (6); (ii) OXA-163, identified from *K. pneumoniae* and *Enterobacter cloacae* isolates in Argentina (7, 8); (iii) OXA-181, identified from a *K. pneumoniae* isolate in India (9); (iv) OXA-204, identified from *K. pneumoniae* isolates in patients having a link with North Africa (10); (v) OXA-232, identified in France from a *K. pneumoniae* isolate recovered from a patient who had been transferred from India to Mauritius (11); (vi) OXA-244 and OXA-245, from *K. pneumoniae* isolates collected in Spain (12); (vii) OXA-247, identified from a *K. pneumoniae* isolate recovered in Argentina (13); and (viii) OXA-370, reported from an *Enterobacter hormaechei* isolate in Brazil (14). These variants differ from OXA-48 by one to five amino acid substitutions and/or by a four-amino-acid deletion which results in a modified β-lactam hydrolysis spectrum.

The epidemiology of carbapenemases worldwide shows that OXA-48 variants are becoming the predominant carbapenemase type in *Enterobacteriaceae* in many regions and countries, such as North Africa, Turkey, France, Germany, and the Middle East.

The aim of this study was to characterize the peculiar molecular mechanisms of resistance to β-lactams, involving a switch from a carbapenem resistance/expanded-spectrum cephalosporin susceptibility profile to a carbapenem susceptibility/expanded-spectrum cephalosporin resistance profile, among two successive *Serratia marcescens* isolates from the same patient.

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TABLE 1 Primers used for mapping of plasmids carrying bla_{OXA-48}-type genes

	Nucleotide data according to GenBank accession no. JN626286				
Primer name	Start	Stop	5′ to 3′ sequence	Location	Amplicon size (bp)
C1F	57425	57444	ATCCGGTCCCCCTGATTATC	IncL/M replicase	4531
C1R	55	74	GTCTGCGACTGACAGACGAT	*trbA*	
C2F	1208	1227	CGAAAGCCAAACCACATCAC	*trbA*	4469
OXA-48-3′external	5655	5676	TATTGTCAAACAAGCCATGCTG	bla_{OXA-48}	
OXA-48-5′external	6099	6119	ATTCCAGAGCACAACTACGCC	bla_{OXA-48}	3025
C3R	9104	9123	CCGTCGTTGTTGCTGAGAAC	*mucB*	
C4F	10248	10267	CGCAGTGGAAGGATATTCCC	*mucB*	4077
C4R	15005	15024	TTCAGGGCGCTGGATTCAAG	*orf12*	
C5F	15480	15499	GCGTGACCGCCTCAAATTCT	*orf12*	4207
C5R	19667	19686	CGAGCACTTACGGTTATCAG	*parB*	
C6F	20083	20102	CATCTGTTCCCGGATGATGA	*parB*	3892
C6R	23955	23974	TCTATGCCGCCCTGTATTCC	*orf25*	
C7F	25154	25173	CAGTGAAGGACTGAGCCACT	*orf25*	4240
C7R	29374	29393	GGCGGGTTGATTCAGTTCAG	*klcA*	
C8F	29786	29805	GATTTACCGCGCGATTGACT	*klcA*	3757
C8R	33523	33542	GACTTTTTGTCCCTTCGGCC	*mobA*	
C9F	35370	35389	GCAGGCGTATGCTCAAAACG	*mobA*	2913
C9R	38263	38282	ACGTTGGCGATCGTCAAAGG	*pri*	
C10F	41356	41375	CAGCCTCAGCATTTACAAGC	*pri*	4613
C10R	45949	45968	TCAGCAGGCTTAGCAGACAC	*traP*	
C11F	46577	46596	CAAGTAAAGGCCTTATCCGC	*traP*	4597
C11R	51154	51173	CTGACCGTTTTGCTTTTCCG	*traW*	
C12F	52321	52340	GAGTGTGAACGCGGGAGTAT	*traW*	4144
C12R	56445	56464	ATGAACTCCGGCGAAAGACC	IncL/M replicase	

MATERIALS AND METHODS

Bacterial strains. Identification of clinical isolates was performed by using the API20E system (bioMérieux, La Balme-les-Grottes, France) and confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MALDI Biotyper CA system, Bruker Daltonics, Billerica, MA, USA). *Escherichia coli* TOP10 (Invitrogen, Saint-Aubin, France) was used for cloning experiments, and azide-resistant *E. coli* J53 was used for conjugation assays.

Susceptibility testing. Antimicrobial susceptibilities were determined by the disk diffusion technique on Mueller-Hinton agar (Bio-Rad, Marnes-La-Coquette, France) and interpreted according to the EUCAST breakpoints, updated in 2014 (http://www.eucast.org). MICs were determined using the Etest technique (bioMérieux).

Detection of carbapenemase activity. The carbapenemase activity was searched for using two techniques: the updated Carba NP test (15) and UV spectrophotometry (16). The updated Carba NP test, which detects imipenemase activity, was performed after plating the culture on a Trypticase soy agar medium supplemented with $ZnSO_4$, as previously described (17). The UV spectrophotometry technique used has been detailed elsewhere (16).

PCR, cloning experiments, and DNA sequencing. Whole-cell DNAs of the two *S. marcescens* isolates and of OXA-48-producing and OXA-163-producing *K. pneumoniae* isolates (8) were extracted using the QIAamp DNA minikit (Qiagen, Courtaboeuf, France) and were then used as a template to amplify the bla_{OXA-48}-like genes. The PCR, using the primers preOXA-48A (5′-TATATTGCATTAAGCAAGGG-3′) and preOXA-48B (5′-CACACAAATACGCGCTAACC-3′), was able to amplify bla_{OXA-48}, $bla_{OXA-163}$, and $bla_{OXA-405}$ genes. The amplicons obtained were then cloned into the pCR-Blunt II-Topo plasmid (Invitrogen) downstream from the pLac promoter, in the same orientation. The recombinant pTOPO-OXA plasmids were electroporated into the *E. coli* TOP10 strain. Plasmid DNAs extraction was performed using the Qiagen miniprep kit. Both strands of the inserts of the recombinant plasmids were sequenced using a T7 promoter and M13 reverse primers with an automated sequencer (ABI Prism 3100; Applied Biosystems). The nucleotide sequences were analyzed using software available at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

Plasmid characterization and mating-out assay. Plasmid DNAs of both clinical *S. marcescens* isolates and OXA-163-producing *K. pneumoniae* 6299 were extracted using the Kieser method (18). Plasmids of ca. 154, 66, 48, and 7 kb of *Escherichia coli* NCTC 5019 were used as plasmid size markers. Plasmid DNA was analyzed by agarose gel electrophoresis. Transfer of the β-lactam resistance markers was attempted by liquid mating-out assays at 37°C using *E. coli* J53 as the recipient strain and by electroporation of the plasmid DNA suspension of clinical isolates into *E. coli* TOP10. Selection of transconjugants was performed on agar-supplemented plates with ticarcillin (100 mg/liter) and with azide (100 mg/liter). Plasmids were typed using the PCR-based replicon typing (PBRT) scheme, as described previously (19), and using the specific primers RepA-A (5′-GACATTGAGTCAGTAGAAGG-3′) and RepA-B(5′-CGTG CAGTTCGTCTTTCGGC-3′) designed for the detection of the IncL/M OXA-48 plasmid replicase (20).

The $bla_{OXA-405}$-carrying plasmid was characterized by PCR mapping followed by DNA sequencing. Fourteen primer pairs were used for the mapping of the 61,881-bp IncL/M plasmid carrying the bla_{OXA-48} gene (Table 1). The bla_{OXA-48}-carrying plasmid sequence (GenBank accession number JN626286) was used as a positive control for PCR mapping (20).

Hydrolysis analysis. The specific activities of the β-lactamases OXA-48, OXA-163, and OXA-405 were determined using the supernatant of a whole-cell crude extract obtained from an overnight culture of *E. coli* clones expressing those β-lactamases (pTOPO-OXA-48, pTOPO-OXA-163, and pTOPO-OXA-405 in *E. coli* TOP 10) with the UV spectrophotometer Ultrospec 2000 (Amersham Pharmacia Biotech), as previously described (10).

Nucleotide sequence accession number. The nucleotide sequence of the $bla_{OXA-405}$ gene has been submitted to the EMBL/GenBank nucleotide sequence database under the accession number KM589641.

TABLE 2 MICs of β-lactams for *S. marcescens* OXA-48 (Sm1), *S. marcescens* OXA-405 (Sm2), *E. coli* pTOPO-OXA-48, *E. coli* pTOPO-OXA-405, *E. coli* pTOPO-OXA-163, and *E. coli* TOP10

	MIC (mg/liter) of:					
β-Lactams	*S. marcescens* OXA-48 (Sm1)	*S. marcescens* OXA-405 (Sm2)	*E. coli* TOP10 (pTOPO-OXA-48)	*E. coli* TOP10 (pTOPO-OXA-405)	*E. coli* TOP10 (pTOPO-OXA-163)	*E. coli* TOP10
Amoxicillin	>256	>256	>256	>256	>256	2
Amoxicillin + CLA[a]	>256	>256	192	>256	96	2
Piperacillin	>256	>256	128	>256	>256	1.5
Piperacillin + TZB[b]	96	>256	12	24	32	1
Temocillin	>256	8	>256	32	32	4
Ticarcillin	>256	>256	>256	>256	>256	2
Cefalotin	>256	>256	8	32	64	2
Cefepime	0.25	3	0.032	0.5	0.5	0.023
Cefepime + TZB[b]	0.25	2	0.032	0.19	0.19	0.023
Cefotaxime	1.5	6	0.19	0.5	3	0.06
Cefotaxime + TZB[b]	1.5	4	0.19	0.19	1	0.06
Ceftazidime	0.25	4	0.25	3	16	0.12
Ceftazidime + TZB[b]	0.25	2	0.25	1	3	0.12
Imipenem	4	0.5	0.5	0.25	0.25	0.19
Meropenem	4	0.19	0.094	0.023	0.023	0.01
Ertapenem	>32	0.75	0.25	0.032	0.032	0.06
Doripenem	3	0.125	0.064	0.023	0.023	0.023
Aztreonam	0.125	4	0.064	1	2	0.047

[a] CLA, clavulanic acid at a fixed concentration of 4 mg/liter.
[b] TZB, tazobactam at a fixed concentration of 4 mg/liter.

RESULTS

Patient features and characteristics of the *S. marcescens* clinical isolates. In January 2011, a 26-year-old woman was admitted at the emergency unit of the University Hospital of Besançon (eastern part of France) for an acute pulmonary infection. After 2 days of hospitalization, blood cultures and a tracheal aspirate identified *S. marcescens* isolates with identical antibiotic susceptibility profiles (these isolates were denoted Sm1). They were resistant to ticarcillin, ticarcillin-clavulanic acid, piperacillin-tazobactam, and temocillin (MIC, >256 mg/liter), had decreased susceptibility to carbapenems (imipenem, meropenem, ertapenem, and doripenem), and remained susceptible to expanded-spectrum cephalosporins (Table 2). A positive Carba NP test indicated the expression of a carbapenemase, and PCR experiments were carried out on purified DNA of Sm1 with primers specific to common carbapenemase genes (*bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{NDM}$, and *bla*$_{OXA-48}$). A *bla*$_{OXA-48}$-like gene was amplified and was later identified as *bla*$_{OXA-48}$ according to sequencing results. The patient was successfully treated with cefepime and amikacin for 15 days. Furthermore, due to the irradiation of the nasopharynx for a carcinoma at the age of 14, the patient presented with important locoregional sequelae composed of sclerosis of the thorax and cervical regions and the persistence of a right laryngeal-cervical fistula. More than 18 months later (October 2012), another *S. marcescens* strain (Sm2) was isolated from a breast hematoma. This *S. marcescens* isolate was resistant to ticarcillin, ticarcillin-clavulanic acid, and piperacillin-tazobactam, had a decreased susceptibility to ertapenem, but remained susceptible to the other tested carbapenem molecules (imipenem, meropenem, and doripenem). The Carba NP test did not reveal carbapenemase activity. Unlike isolate Sm1, isolate Sm2 was resistant to expanded-spectrum cephalosporins (cefotaxime, ceftazidime, cefepime) and aztreonam (Table 2) and recovered susceptibility to temocillin (MIC, 8 mg/liter). PCR using whole-cell DNA of Sm2 as the template was positive for a *bla*$_{OXA-48}$-like gene. Sequencing results identified a novel *bla*$_{OXA-48}$-like gene, designated the *bla*$_{OXA-405}$ gene.

Genomic comparison using a Rep-PCR-based technique (DiversiLab, bioMérieux) identified a 97.8% genomic similarity between the *S. marcescens* Sm1 and Sm2 isolates (Fig. 1A). Therefore, these strains were considered to be clonally related. This clonality has been confirmed by pulsed-field gel electrophoresis (Fig. 1B).

Characterization of the β-lactamase OXA-405. This *bla*$_{OXA-405}$ gene differs from the *bla*$_{OXA-48}$ gene by a 12-bp deletion leading to a four-amino-acid deletion in the OXA-405 protein sequence from residues Thr213 to Glu216 compared to the OXA-48 sequence (Fig. 2). The comparison of the hydrolysis spectra of OXA-405, OXA-48, and OXA-163 was done by cloning *bla*$_{OXA-405}$, *bla*$_{OXA-48}$, and *bla*$_{OXA-163}$ genes in the pCR-Blunt II-Topo kit (Invitrogen) and expressing them in *E. coli* TOP10. OXA-405 and OXA-163 conferred similar resistance profiles, consisting of a decreased susceptibility to expanded-spectrum cephalosporins and aztreonam compared to that conferred by OXA-48 (Table 2). As opposed to OXA-48, OXA-405, like the OXA-163 enzyme, once expressed in a reference *E. coli* strain was not associated with a decreased susceptibility to carbapenems (Table 2). Both the Carba NP test and the UV spectrophotometry analysis showed that OXA-405 and OXA-163 did not express significant imipenemase activity (Table 3). In addition, OXA-405 producers and also OXA-163 producers were 8-fold more susceptible to temocillin than OXA-48 producers (Table 2).

The specific activities of OXA-405 and of OXA-163 were very similar for penicillins, broad-spectrum cephalosporins, and carbapenems. However, OXA-405 hydrolyzed less ceftazidime (6-fold less) than OXA-163 (Table 3). Both OXA-405 and OXA-163 had barely detectable activity against carbapenems compared to OXA-48 (~25-fold less for imipenem) (Table 3). On the other hand, OXA-405 and OXA-163 hydrolyzed expanded-spectrum cephalosporins and aztreonam at high rates, while OXA-48 did

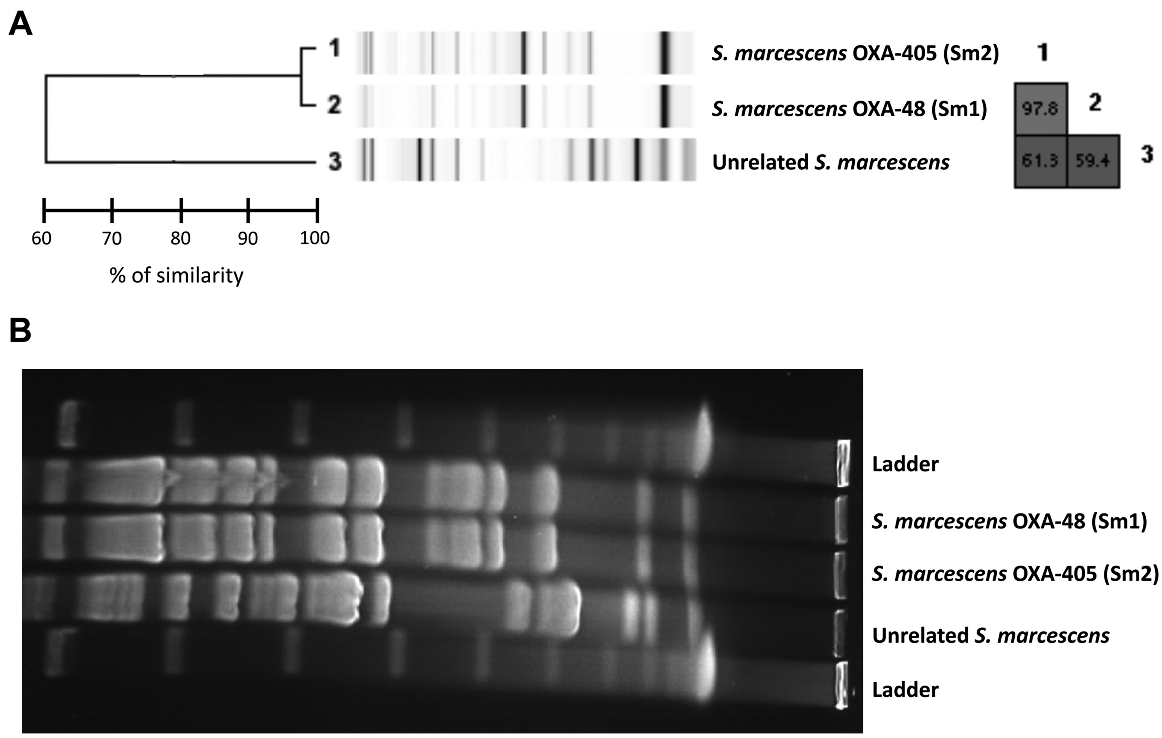

FIG 1 (A) Rep-PCR analysis using the Diversilab technique. Dendrogram and computer-generated image of Rep-PCR banding patterns of OXA-48-producing *S. marcescens*, OXA-405-producing *S. marcescens*, and an unrelated strain of *S. marcescens*. (B) Pulsed-field gel electrophoresis of OXA-48-producing *S. marcescens*, OXA-405-producing *S. marcescens*, and an unrelated strain of *S. marcescens*.

not (Table 3). This activity against expanded-spectrum cephalosporins of OXA-405 was inhibited by the addition of tazobactam (Table 2).

Genetic environment of the *bla*$_{\mathbf{OXA\text{-}405}}$ gene. The *bla*$_{OXA\text{-}405}$ gene was located on transposon Tn*1999*, as the *bla*$_{OXA\text{-}48}$ gene usually is (4, 20). Plasmid DNAs of *S. marcescens* Sm1 (pOXA-48) and Sm2 (pOXA-405) were extracted and compared. A single plasmid was identified from each strain, of ca. 62 kb and ca. 46 kb for Sm1 and Sm2, respectively. A PCR-based replicon typing method revealed that these plasmids belonged to the same IncL/M incompatibility group. Whereas transformants in *E. coli* were obtained by using both plasmids, transconjugants were obtained with the pOXA-48 plasmid only. PCR mapping of plasmids pOXA-48 and pOXA-405 showed that pOXA-48 was structurally identical to the prototype IncL/M OXA-48-positive plasmid. Plasmid pOXA-405 had a backbone similar to that of pOXA-48 but had a 16,382-bp deletion from nucleotides 24210 to 40587 according to the reference *bla*$_{OXA\text{-}48}$ plasmid (number JN626286, GenBank nucleotide database) (20). This deletion included the *ssb* gene, the *mobC* and *mobA* genes, the *nikB* and *nikA* genes, and a part of locus *tra* (*traH*, *traI*, *traJ*, *traK*, *traL*, and primase genes). This deleted DNA section was replaced by an insertion sequence, IS*1R* (Fig. 3B).

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          1         10        20        30        40        50        60        70        80        90        100
          |         |         |         |         |         |         |         |         |         |         |
OXA-48    MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR
OXA-405   MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR
OXA-163   MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR
OXA-247   MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR

                    110       120       130       140       150       160       170       180       190       200
                    |         |         |         |         |         |         |         |         |         |
OXA-48    DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN
OXA-405   DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN
OXA-163   DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN
OXA-247   DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN

                    210       220       230       240       250       260
                    |         |         |         |         |         |
OXA-48    GDYIIRAKTGYSTRIEPKKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
OXA-405   GDYIIRAKTGYS----PKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
OXA-163   GDYIIRAKTGYDT----KIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
OXA-247   GDYIIRAKTGSNT----KIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
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FIG 2 Alignment of the amino acid sequences of OXA-48, OXA-405, OXA-163, and OXA-247. Possible conserved residues of the active site of the OXA-48-type β-lactamases are highlighted in gray.

TABLE 3 Specific activities of β-lactamases OXA-48, OXA-405, and OXA-163

β-Lactams	Specific activity (mean mU/mg of protein ± SD) of:		
	OXA-48	OXA-405	OXA-163
Amoxicillin	981 ± 62	485 ± 35	795 ± 81
Piperacillin	450 ± 5	436 ± 4	214 ± 2
Temocillin	11 ± 2	5 ± 0.5	5 ± 0.4
Ticarcillin	647 ± 59	63 ± 6	80 ± 7
Cefepime	5 ± 0.5	27 ± 2	30 ± 3
Cefotaxime	60 ± 6	117 ± 10	167 ± 15
Cefoxitin	2 ± 0.2	1 ± 0.1	1 ± 0.1
Ceftazidime	2 ± 0.2	9 ± 0.8	53 ± 5
Cefalotin	75 ± 8	140 ± 12	130 ± 10
Imipenem	57 ± 4	3 ± 0.2	2 ± 0.2
Meropenem	3 ± 0.1	2 ± 0.2	2 ± 0.1
Ertapenem	2 ± 0.2	1 ± 0.1	1 ± 0.1
Doripenem	2 ± 0.2	1 ± 0.1	1 ± 0.1
Aztreonam	5 ± 0.5	14 ± 1	18 ± 2

DISCUSSION

A novel OXA-48-type β-lactamase, OXA-405, has been identified here. OXA-405, like the other OXA-48-type β-lactamases OXA-163 and OXA-247, has a significant activity toward expanded-spectrum cephalosporins but barely any activity toward carbapenems. Therefore, it should be underlined that OXA-48-like β-lactamases, as opposed to all known KPC, NDM, VIM, or IMP β-lactamases, are not all significant carbapenemases. In addition, it has been shown that OXA-48-type producers with carbapenemase activity are mostly resistant to temocillin. Here, we confirm that this temocillin resistance trait would be a good criterion for differentiating OXA-48-type producers with and without carbapenemase activity.

Structural protein analysis of OXA-405, OXA-163, and OXA-247 showed that they possess at least the same four-amino-acid deletion in a specific region, from Thr213 to Glu216 (8, 13). This result agrees with crystal structure analysis of OXA-48 showing that Arg 214 (which is part of a β5 strand) is critical for carbapenemase activity (21). In addition, recent studies point out the crucial role of this short loop connecting β5 and β6 strands in conferring carbapenemase activity of Ambler class D β-lactamases (22, 23).

Genetic analysis of the *S. marcescens* clinical isolates Sm1 and Sm2 producing OXA-48 and OXA-405, respectively, indicates that they are clonally related. This result suggests that the $bla_{\text{OXA-405}}$ gene may derive from the same ancestor, a $bla_{\text{OXA-48}}$ gene. This hypothesis is reinforced by the common genetic environment of those two genes. Actually, the $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-405}}$ genes were

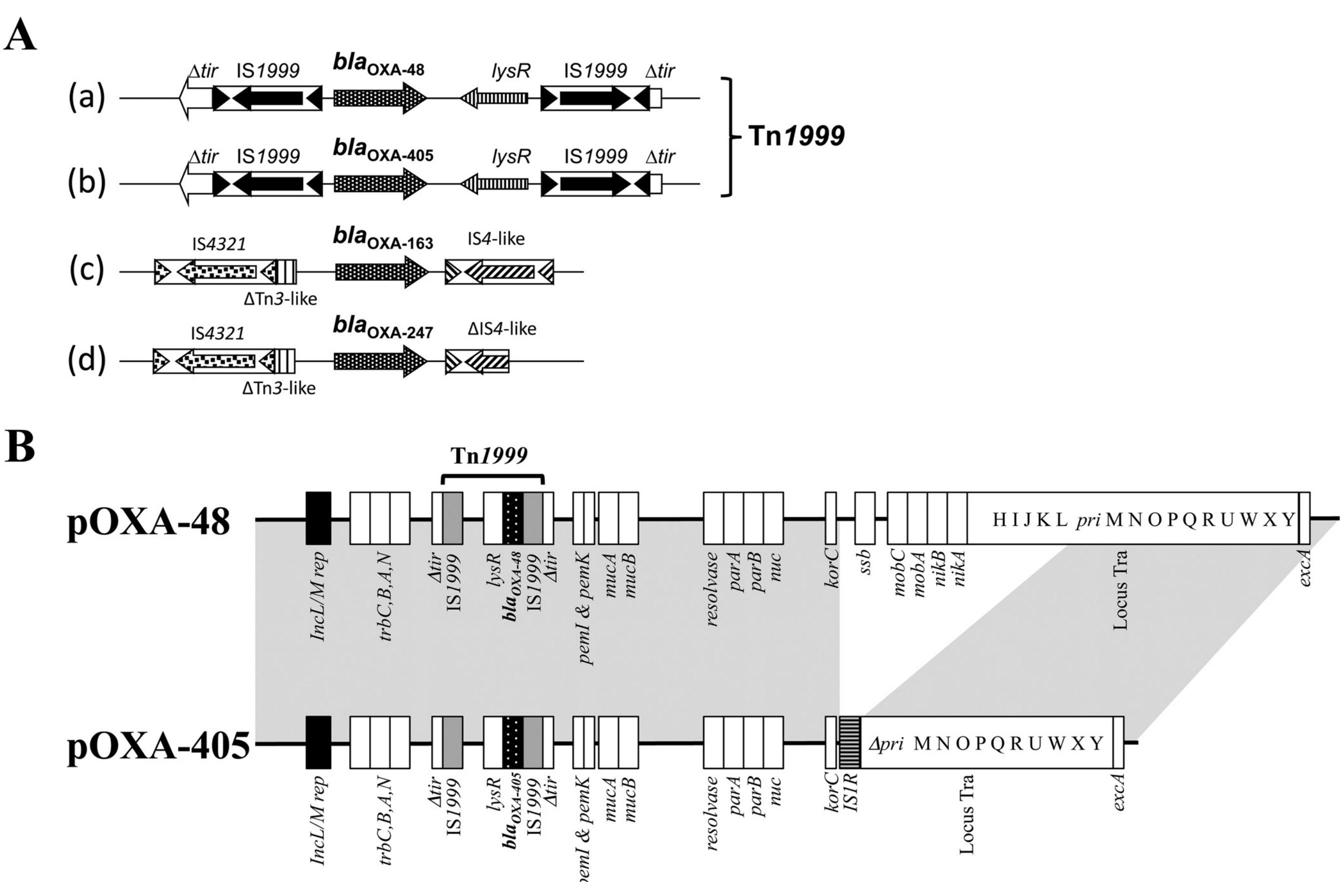

FIG 3 (A) Schematic representation of the genetic environment of the $bla_{\text{OXA-48}}$ (a), $bla_{\text{OXA-405}}$ (b), $bla_{\text{OXA-163}}$ (c), and $bla_{\text{OXA-247}}$ (d) genes. The Tn*1999* composite transposon is made of two copies of insertion sequence IS*1999*, bracketing a fragment containing the $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-405}}$ genes. (B) Major structural features of the plasmid pOXA-405 from *S. marcescens* Sm2 in comparison with the prototype IncL/M $bla_{\text{OXA-48}}$ plasmid (pOXA-48) (GenBank accession number JN626286). Common structures are highlighted in gray.

bracketed by two copies of an identical IS element, IS*1999*, forming a composite transposon Tn*1999*. This genetic environment was completely different from the mosaic structures made of insertion sequences and the truncated mobile element that surrounds the $bla_{OXA-163}$ gene and its derivative, $bla_{OXA-247}$ (Fig. 3) (8, 13). In addition, the $bla_{OXA-405}$ gene was identified on the plasmid pOXA-405, which possessed a backbone similar to that of the IncL/M bla_{OXA-48}-bearing plasmid (pOXA-48) (20), except for a deletion of ca. 16 kb replaced by the insertion sequence IS*1R*. This deletion/insertion led to the loss of conjugative genes and the related self-conjugative property of pOXA-405 (20). The selection pressure of a cephalosporin-containing treatment (here, cefepime) remains to be determined for selecting an OXA-48-type β-lactamase with activity against expanded-spectrum cephalosporins from an OXA-48-type β-lactamase with carbapenemase activity.

In conclusion, this report underlines that OXA-48-type β-lactamases are more diverse than expected. As exemplified by OXA-405, the OXA-48-type β-lactamases are not all true carbapenemases. The same statement is valid for another group of serine β-lactamases, the GES group of enzymes, in which GES-1 is an extended-spectrum β-lactamase, while GES-2 is a carbapenemase (24). Therefore, the first-line screening of carbapenemase producers in *Enterobacteriaceae* may be best based on the biochemical detection of carbapenemase activity in clinical settings. The molecular biology techniques, although useful, may overreport OXA-48-like producers as being all carbapenemases and, conversely, may fail to detect carbapenemase producers related to totally novel or slightly structurally modified carbapenemase genes.

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